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## Circulating Secretin Activates Supraoptic Nucleus Oxytocin and Vasopressin Neurons via Noradrenergic Pathways in the Rat

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Secretin is a 27-amino acid brain-gut peptide from duodenal S-cells. We tested the effects of systemic administration of secretin to simulate its postprandial release on neuroendocrine neurons of the supraoptic nucleus (SON) in urethane-anesthetized female rats. Secretin dose-dependently increased the firing rate of oxytocin neurons, more potently than cholecystokinin, and dose-dependently increased plasma oxytocin concentration. The effect of secretin on SON vasopressin neurons was also predominantly excitatory, in contrast to the inhibitory actions of cholecystokinin. To explore the involvement of noradrenergic inputs in secretin-induced excitation, benoxathian, an  $\alpha 1$ -adrenoceptor antagonist, was infused intracerebroventricularly. Benoxathian intracerebroventricular infusion blocked the excitation by secretin of both oxytocin and vasopressin neurons. To test the role of local noradrenaline release in the SON, benoxathian was microdialyzed onto the SON. The basal firing rate of oxytocin neurons was slightly reduced and the secretin-induced excitation was attenuated during benoxathian microdialysis. Hence, noradrenergic pathways mediate the excitation by systemic secretin of oxytocin neurons via  $\alpha 1$ -adrenoceptors in the SON. As both systemic secretin and oxytocin are involved in regulating gastrointestinal functions and natriuresis, systemically released secretin might act partly through oxytocin. (*Endocrinology* 151: 2681–2688, 2010)

Secretin, a peptide hormone synthesized by the duodenal S-cells, is secreted into the systemic circulation when acidic contents enter the duodenum from the stomach. Secretin acts locally to neutralize this acidity by stimulating the secretion of fluid and bicarbonate ions from the pancreas (1, 2), and also promotes the secretion of bile from the liver. In addition, secretin may also signal to the CNS, as peripheral injections of secretin result in Fos expression in specific brain areas (3, 4), including the supraoptic nucleus (SON) of the hypothalamus (4), which contains the cell bodies of magnocellular neuroendocrine neurons that project to the posterior pituitary gland to secrete oxytocin and vasopressin. Intracerebroventricular (i.c.v.) administration of secretin also increases Fos expression in SON neurons and increases peripheral secretion of oxytocin and vasopressin, and secretin receptors

are found in the SON and the magnocellular area of the paraventricular nucleus (5). However, secretin also activates vagal primary afferent neurons (6).

Many gut-derived factors are now known to signal to the hypothalamus, either directly or indirectly via vagal afferents. To test the hypothesis that circulating secretin has a physiological action on the hypothalamus, we used low doses of systemic secretin ( $\leq 1 \mu\text{g}/\text{rat}$ ) (7), and studied their effects on the electrical activity of neuroendocrine neurons in the SON *in vivo*. We compared the responses to secretin with responses to cholecystokinin (CCK), a functionally similar peptide (8). When administered iv, CCK excites oxytocin neurons and either inhibits or does not affect the firing rate of SON vasopressin neurons (9–11).

The SON receives excitatory noradrenergic inputs from the nucleus tractus solitarii (NTS) (12). The activa-

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Abbreviations: AP, Area postrema; aCSF, artificial cerebrospinal fluid; CCK, cholecystokinin; i.c.v., intracerebroventricular; NTS, nucleus tractus solitarii; RM, repeated measures; SON, supraoptic nucleus.

tion of  $\alpha 1$  adrenergic receptors induces release of oxytocin and vasopressin from hypothalamic explants (13). To investigate the hypothesis that excitatory noradrenergic pathways mediate secretin-induced responses in SON neurons via  $\alpha 1$  adrenergic receptors, the effect on the responses to secretin of i.c.v. infusion and microdialysis application to the SON of benoxathian hydrochloride (a competitive  $\alpha 1$  adrenergic antagonist) was also studied.

## Materials and Methods

### Animals

Adult ( $\geq 10$  wk old) female Sprague Dawley rats of 200–300 g body weight were obtained from the colony maintained at the University of Edinburgh. The rats were housed under a 12-h light, 12-h dark cycle (lights on at 0700 h) with free access to food and water. Temperature was maintained at 19–23 C and humidity at  $55 \pm 10\%$ . All procedures were performed in accordance with the current United Kingdom Home Office regulations under the Animals (Scientific Procedures) Act 1986.

### In vivo electrophysiology

Anesthesia was induced by brief exposure to halothane vapor in an anesthetic chamber, followed by ip injection of urethane (ethyl carbamate; 25% wt/vol; 1.25 g/kg). The left femoral vein was cannulated for iv drug administration and connected to a syringe containing 0.9% NaCl. An endotracheal tube was inserted into the trachea to keep the airway patent. Using a ventral transpharyngeal surgical procedure (14), the SON and neural stalk were exposed. A glass recording microelectrode with a tip diameter of approximately 1  $\mu\text{m}$  filled with 0.15 M NaCl was lowered into the SON under visual control. Signals were amplified and 50 Hz noise and harmonics were eliminated from the signal (Hum Bug, Quest Scientific, Vancouver, British Columbia, Canada). A Digitimer D130 spike processor was used to detect spikes, and the raw signal and the detected spikes were passed through an interface (CED1401; Cambridge Electronic Design, Cambridge, UK) to a personal computer for analysis using Spike2 software (CED). In each rat, between one and three neurons were recorded and tested with injections of secretin. At the end of each experiment, rats were killed by an overdose of sodium pentobarbitone (60 mg/kg; iv), and correct placement of any i.c.v. cannula confirmed.

SON neurons were antidromically identified as projecting to the posterior pituitary by electrical stimulation of the neural stalk (matched biphasic pulses, 1 mA for 1 msec, via a bipolar stimulating electrode). Oxytocin neurons were differentiated from vasopressin neurons by their firing pattern; many vasopressin cells fire “phasically,” *i.e.*, in prolonged bursts of action potentials separated by long silent phases. However, other vasopressin neurons fire continuously, and oxytocin neurons were differentiated from these by the shape of their interspike interval histogram and hazard function (15). For oxytocin neurons, but not for vasopressin neurons, the descending tails of the interspike interval distributions can be fitted well using a single negative exponential (16). Hazard functions plot how the excitability of a cell changes with time after it has fired a spike. Specifically, hazard functions show the probability of occurrence of a spike as a

function of the time since the last spike; oxytocin neurons show a constant hazard after a postspike interval (refractory period) of about 50 msec, implying that after this there are no detectable effects of an individual spike upon subsequent neuronal excitability (15). Ratios of peak early ( $<0.07$ -sec interspike interval) to mean late (0.2- to 0.3-sec interspike intervals) hazards were calculated before and after secretin administration to seek change in the hazard plot as an indicator of altered excitability.

The basal firing rate of neurons was recorded for 10 min, after which the rats were injected with iv CCK (sulfated cholecystokinin octapeptide; 25  $\mu\text{g/kg}$ ; Tocris Bioscience, Bristol, UK): oxytocin neurons are transiently excited by CCK whereas vasopressin neurons are mostly transiently inhibited or are unaffected (9). Then secretin (Tocris Bioscience) was administered iv at doses of 0.01, 0.1, or 1  $\mu\text{g}$  in 100  $\mu\text{l}$  normal saline. Vasopressin neurons were studied using a single dose of secretin (0.1  $\mu\text{g/rat}$ ) and responses were compared with responses to iv CCK (25  $\mu\text{g/kg}$ ).

To study the involvement of noradrenergic inputs, benoxathian hydrochloride (Sigma, Dorset, UK), dissolved in artificial cerebrospinal fluid [aCSF; composition: NaCl, 138 mM; KCl, 3.36 mM;  $\text{NaHCO}_3$ , 9.52 mM;  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , 0.49 mM; urea, 2.16 mM;  $\text{CaCl}_2$ , 1.26 mM;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 1.18 mM (pH: 7.2); 300 mOsm], was infused i.c.v. (8  $\mu\text{g}/\mu\text{l min}^{-1}$ ), and the responses to secretin before and during the infusion were compared. In further experiments, benoxathian was microdialyzed (2 mM) onto the ventral surface of the SON.

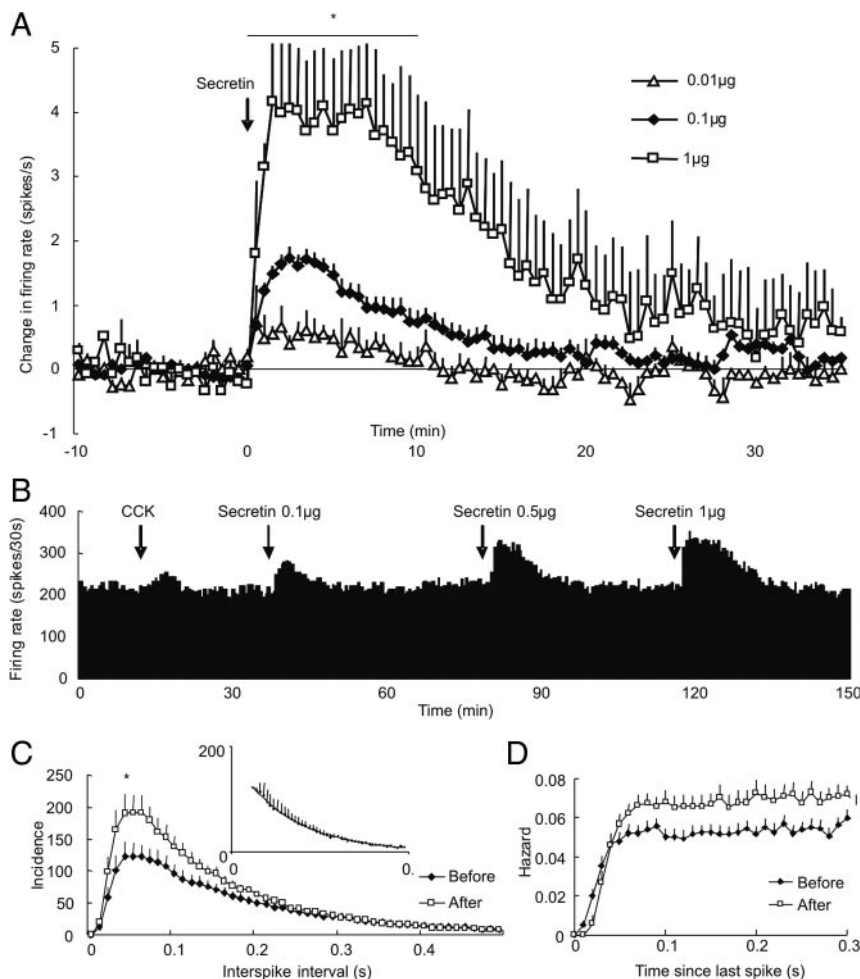
Firing rates were averaged in 30-sec bins. For nonphasic oxytocin and vasopressin neurons, the mean change in firing rate (spikes/sec) was calculated for each 30-sec bin from the basal activity (over 10 min) and activity after each injection. Phasic firing patterns were evaluated by calculating the activity quotient (the ratio of period active to total period), the frequency within bursts (mean number of spikes per burst over mean burst length), and the mean interburst interval.

### Blood sampling

The left femoral vein was cannulated and connected to a syringe containing heparinized 0.9% saline (50 U/ml) in urethane-anesthetized rats. At least 2 h later, a basal blood sample (0.3 ml) was taken into a heparinized syringe (30  $\mu\text{l}$  of 50 U/ml heparin in 0.9% saline), followed by a vehicle injection (saline; 100  $\mu\text{l}$ ; iv), followed by a second sample 5 min later; withdrawn blood was immediately replaced with an equivalent volume of sterile 0.9% saline, iv. Samples were collected 5, 10, and 25 min after 0.1  $\mu\text{g}$  iv secretin and 5 and 10 min after 1  $\mu\text{g}$  iv secretin. Plasma was separated and stored at  $-20$  C until assayed.

### Plasma oxytocin RIA

This method was adapted from that of Higuchi *et al.* (17). The primary antibody THF-3 (rabbit anti-oxytocin), kindly supplied by Takashi Higuchi (University of Fukui, Fukui, Japan), was diluted to a final concentration of 1:200,000 in RIA buffer and added to tubes containing standard oxytocin (National Institute for Biological Standards and Controls, Hertfordshire, UK; range 2.4–2500 pg/ml; in triplicate) or test samples (in duplicate). After 24 h incubation at 4 C, radioiodinated oxytocin ( $^{125}\text{I}$ , 3.7 KBq/ml, 50  $\mu\text{l}$ ; PerkinElmer, Life and Analytical Sciences, Buckinghamshire, UK) was added to all of the tubes. After 48-h incubation at 4 C, a donkey antirabbit antibody (IDS Ltd., Boldon, UK) at a dilution of 1:25 in assay buffer was added and incubated overnight at 4 C. The following day, the antigen-antibody com-



**FIG. 1.** Effect of secretin on SON oxytocin neurons. Extracellular recordings of firing rates in urethane-anesthetized female rats. A, Dose-dependent responses of oxytocin neurons to increasing doses per rat of iv secretin at 1 µg:  $n = 3$ ; 0.1 µg:  $n = 26$  (all cells in this dose-effect and subsequent experiments); and 0.01 µg:  $n = 4$ . Data are group means  $\pm$  SEM;  $n$  = number of cells/rats. \*,  $P < 0.001$ , one-way ANOVA, 0–10 min postsecretin among groups; pre- vs. 0–10 min postsecretin within the 1 µg group:  $P = 0.04$ , paired  $t$  test; pre- vs. 0–10 min postsecretin within the 0.1 µg group:  $P < 0.001$ , paired  $t$  test. B, Representative dose-dependent responses of an oxytocin neuron to secretin. CCK (25 µg/kg) and 0.1 µg, 0.5 µg, and 1 µg secretin were given iv as indicated by the arrows. C, Interspike interval histograms of neurons shown in A (secretin 0.1 µg): \*,  $P < 0.001$ , paired  $t$  test, incidence of interspike intervals less than 0.5 sec 0–10 min after secretin vs. 10 min before secretin ( $n = 26$ ). The descending slope of the histogram was fitted well with an exponential curve ( $y = 177.56e^{-6.2398x}$ ) that characterizes oxytocin neurons (inset);  $r^2 = 0.996$ . D, Hazard analysis of firing of neurons shown in A (0.1 µg;  $n = 26$ ). Despite a significant positive shift in the hazard plot ( $P < 0.001$ , Wilcoxon signed rank test), the profile of activity-dependent changes in excitability was similar before and after secretin.

plex was precipitated by adding standardized Pansorbin cells diluted to 1:25 in assay buffer. Radioactivity of the precipitate was measured with a  $\gamma$ -counter (Wizard 1470 Automatic  $\gamma$  Counter, PerkinElmer, Life and Analytical Sciences) and unknown oxytocin concentrations were read directly from the standard curve constructed automatically by the integrated data analysis software (Wizard). The sensitivity of the assay was 2.4 pg/ml, and the intraassay variation was less than 15%.

### Intracerebroventricular cannulation

A guide cannula (22 G; Bilaney Consultants Ltd., Kent, UK) was positioned 1.6 mm lateral and 0.6 mm caudal to bregma

with its tip 3.5 mm below the skull surface in urethane-anesthetized rats. A 28-G internal cannula (Bilaney Consultants Ltd.) that projected 1 mm below the tip of the guide cannula was used for administration of benoxathian into the right lateral cerebral ventricle (i.c.v.).

### In vivo microdialysis

As described above, rats were anesthetized and the ventral surface of the brain was exposed. A U-shaped microdialysis probe was prepared in house (membrane length 2 mm; inner diameter 200 µm; mean pore diameter: 4–6 nm; Cuprophane RC55, Membrana, GmbH, Wuppertal, Germany). The loop of the probe was positioned on the ventral surface of the SON after opening the meninges. The recording electrode was positioned within the loop (18). The SON was microdialyzed at 3 µl/min with aCSF or benoxathian (2 mM in aCSF) from a microsyringe mounted on a slow infusion pump.

### Statistical analysis

Data are shown as means  $\pm$  SEM. Statistical tests were performed with SigmaStat software version 3.10 (Systat Software Inc., London, UK). Data were analyzed by paired  $t$  test,  $t$  test, or one-way or two-way ANOVA followed by appropriate *post hoc* pairwise tests or one-way repeated measures (RM) ANOVA as appropriate. The significance level for all statistics was set at  $P < 0.05$ .

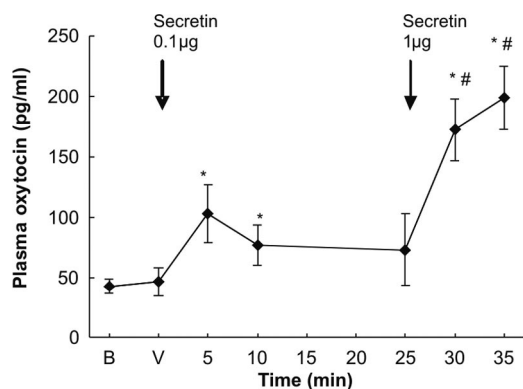
### Results

In initial studies (not shown), we used doses of secretin similar to those that have been used previously (5 and 10 µg/rat; i.v.) (3, 4); these doses elicited strong and prolonged excitatory responses in SON oxytocin and vasopressin neurons, so we gradually reduced the dose in subsequent experiments, until we found that doses below 0.01 µg iv were not consistently effective.

### Effect of secretin on oxytocin neurons

In the range 0.01–1 µg iv, secretin dose dependently increased the firing rate of SON oxytocin neurons (Fig. 1, A and B;  $F = 17.5$ , degrees of freedom = 2,  $P < 0.001$ , one-way ANOVA). An increase in firing rate was apparent within about 30 sec, reaching a maximum within about 2 min and gradually subsiding to the basal firing rate by



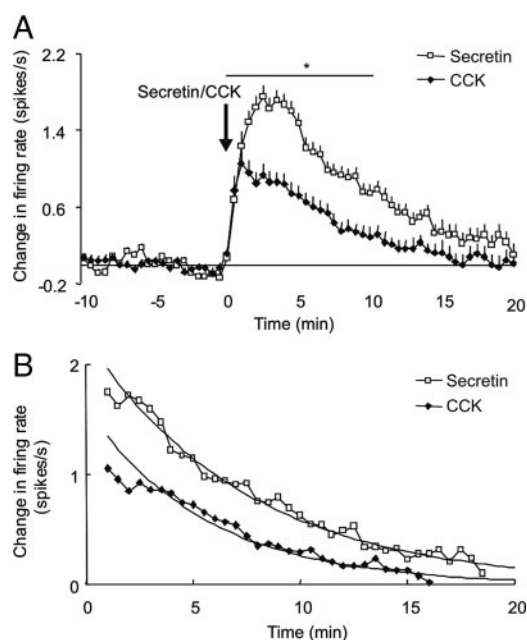


**FIG. 2.** Effect of systemic administration of secretin on systemic oxytocin release in urethane-anesthetized female rats. Plasma oxytocin concentration: A basal sample (B) was collected 2 h after jugular cannulation; a sample (V) was taken 5 min after vehicle (100  $\mu$ l normal saline; iv). Subsequently, samples were collected 5, 10, and 25 min after iv administration of 0.1  $\mu$ g secretin and 5 and 10 min after 1  $\mu$ g secretin. Values are group means  $\pm$  SEM;  $n = 6$  rats per group. \*,  $P < 0.05$  (0.1  $\mu$ g secretin) and  $P = 0.001$  (1  $\mu$ g secretin), one-way RM ANOVA, vs. basal and vehicle control. #,  $P = 0.001$ , one-way RM ANOVA, vs. 5 and 10 min after 0.1  $\mu$ g secretin.

20–30 min. Cells fired continuously throughout activation, with no change in the modal interspike interval (Fig. 1C). There was a significant positive shift in the hazard function after secretin (Fig. 1D;  $P < 0.001$ , Wilcoxon signed rank test) with no change in its overall shape, as indicated by the lack of change in the ratio of peak early ( $<0.07$  sec) to mean late (0.2–0.3 sec) hazard 10 min before ( $1.13 \pm 0.08$ ) and after ( $1.09 \pm 0.08$ ) secretin ( $P = 0.7$ , Wilcoxon signed rank test). There was no correlation between basal firing rate and the increase in the firing rate ( $r^2 = 0.0026$ ). In parallel with the increase in the electrical activity of SON oxytocin neurons, plasma oxytocin concentration was significantly and dose-dependently increased (Fig. 2).

### Response of oxytocin neurons to secretin and CCK

Secretin, even at a low dose (0.1  $\mu$ g/rat; 33 pmol), was more potent than CCK (7.5  $\mu$ g; 6600 pmol; Fig. 3A;  $P < 0.001$ ,  $t$  test). The recovery phase of firing rate after excitation induced by secretin or CCK was fitted well by monoexponential curves (Fig. 3B) of the form  $R_{(t)} = R_0 e^{-\lambda t}$  where  $R_{(t)}$  is the rate at time  $t$  after the peak and  $R_0$  is the rate at the peak of excitation;  $\lambda$  is the decay constant. The half-life for the recovery phase was calculated as  $t_{1/2} = \tau \times \log_2$  where  $\tau$  (tau) is  $1/\lambda$ . Half-life estimates for circulating secretin range from 1.5–5 min (19, 20). Estimated plasma concentrations (assuming distribution in ECF, 60 ml), for a half-life of 5 min (19), immediately and 10 min after injection of 100 ng secretin were 1700 and 425 pg/ml; for a half-life of 1.5 min (20), estimated plasma concentration was approximately 400 pg/ml at 5 min, and less than 100 pg/ml by 10 min. These values are in or near the



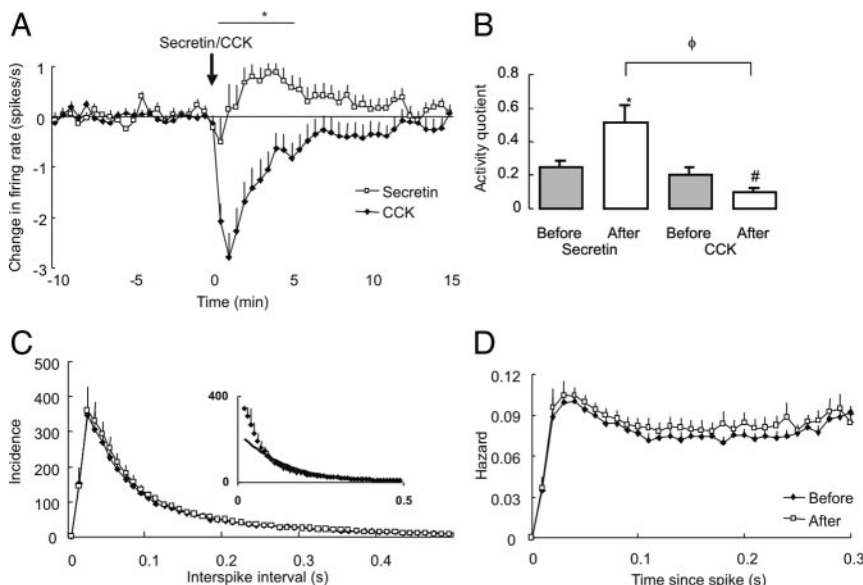
**FIG. 3.** Comparison of secretin and CCK-induced oxytocin neuron responses in urethane-anesthetized female rats. A, Effect of secretin (0.1  $\mu$ g/rat; iv) on the electrical activity of SON oxytocin neurons compared with the CCK-induced (25  $\mu$ g/kg; iv) excitatory response; values are group means  $\pm$  SEM. Group numbers are: secretin:  $n = 26$  neurons/rats; CCK:  $n = 45$  neurons/rats. \*,  $P < 0.001$ ,  $t$  test, secretin vs. CCK 0–10 min after administration between the groups. Pre- vs. 0–10 min postsecretin within the group:  $P < 0.001$ , paired  $t$  test; pre- vs. 0–10 min post-CCK within the group:  $P < 0.001$ , Wilcoxon signed rank test. B, The change in firing rate fitted to exponential curves. Equation for secretin:  $y = 2.2521e^{-0.1353x}$ ; equation for CCK:  $y = 1.6231e^{-0.1829x}$ .  $r^2$  values of 0.95 and 0.89 for secretin and CCK, respectively, indicate a good fit. The half-lives calculated from exponential decreases in the firing rate are 5.1 and 3.8 min, respectively, for secretin and CCK.

physiological range, 70 pg/ml (basal) to 730 pg/ml (during 5 d salt loading) (21). Oxytocin neurones were consistently still strongly excited at 5–10 min after secretin (100 ng) injection (Fig. 3), when secretin concentration was in or near this physiological range.

### Effect of secretin on vasopressin neurons

The responses of the two types of vasopressin neuron, continuous and phasic, are described separately. In response to secretin, 14 of 21 nonphasic vasopressin neurons were excited, six were inhibited and one did not respond; the overall response of this population to secretin was not significant (Fig. 4A;  $P = 0.2$ , paired  $t$  test). By contrast, in response to CCK, 23 of 30 nonphasic vasopressin neurons were inhibited and seven did not respond; the overall population response was a significant inhibition (Fig. 4A;  $P < 0.001$ , Wilcoxon signed rank test).

Systemic administration of secretin excited each of six phasic vasopressin neurons tested, whereas CCK inhibited each of them. Secretin significantly increased and CCK significantly decreased the activity quotient of these neu-



**FIG. 4.** Effect of systemic administration of secretin ( $0.1 \mu\text{g}$ ; iv) and CCK ( $25 \mu\text{g/kg}$ ; iv) on the firing rate of vasopressin neurons in urethane-anesthetized female rats. Values are group means  $\pm$  SEM. **A**, Nonphasic vasopressin neurons. Group numbers are: secretin:  $n = 21$  cells/rats; CCK:  $n = 30$  cells/rats. CCK inhibited firing and secretin excited firing; \*,  $P < 0.001$ ,  $t$  test, 0–5 min after secretin vs. CCK. **B**, Phasic vasopressin neurons: Activity quotient. Group numbers are: secretin:  $n = 11$  cells/rats; CCK:  $n = 7$  cells/rats. Secretin was excitatory and CCK was inhibitory; \*,  $P = 0.02$ , paired  $t$  test, pre- vs. 0–5 min postsecretin; #,  $P = 0.02$ , paired  $t$  test, pre- vs. 0–5 min post-CCK.  $\phi$ ,  $P = 0.004$ , Kruskal-Wallis one-way ANOVA on ranks, secretin vs. CCK response. **C**, Interspike interval histogram: incidence of interspike intervals less than 0.5 sec 0–10 min after secretin ( $0.1 \mu\text{g}$ ) vs. 10-min basal period in vasopressin neurons (combined histogram for phasic and nonphasic neurons;  $n = 34$ ). There was no change in the incidence after secretin:  $P = 0.2$ , paired  $t$  test. *Inset*, The distal tail of the descending slope of the histogram fitted an exponential curve ( $y = 237.29e^{-7.2707x}$ );  $r^2$  value of 0.96 indicates a good fit. However, intervals less than 0.2 sec did not fit: this characterizes vasopressin neurons. **D**, Hazard analysis. There was a significant positive shift in the hazard plot after secretin ( $0.1 \mu\text{g}$ ) administration ( $P = 0.001$ , Wilcoxon signed rank test); however, the profile of activity-dependent changes in excitability was similar before and after secretin.

rons (Fig. 4B). Secretin significantly decreased the mean interburst interval ( $P = 0.01$ , Wilcoxon signed rank test), whereas CCK did not alter the interburst interval, but there was no significant difference between secretin and CCK treatment ( $P = 0.4$ ,  $t$  test). There was no change in the mode of the interspike interval distribution (Fig. 4C) after secretin. There was a positive shift in the hazard function after secretin (Fig. 4D;  $P = 0.001$ , Wilcoxon signed rank test) with no change in its overall shape.

#### Effect of i.c.v. benoxathian on secretin-induced excitation of oxytocin neurons

In control conditions, repeated injections of secretin ( $0.1 \mu\text{g/rat}$ ) produced similar responses with each injection, in three oxytocin neurons (e.g. Fig. 5A) and seven vasopressin neurons tested (e.g. Fig. 5, B and D). In separate experiments, we then tested the effects of i.c.v. benoxathian infusion on responses to this dose of secretin.

Six oxytocin neurons were studied during i.c.v. benoxathian infusion, which reduced their basal firing rate of

$5.7 \pm 1.05$  spikes/sec to  $2.2 \pm 0.9$  spikes/sec ( $P = 0.03$ , paired  $t$  test). The secretin-induced excitatory response was suppressed in all six neurons by i.c.v. benoxathian; responses to iv CCK were similarly blocked (Fig. 5C). Before benoxathian, secretin increased the firing rate by  $1.6 \pm 0.1$  spikes/sec ( $P < 0.001$ , paired  $t$  test); during benoxathian infusion, secretin had no effect (Fig. 5E;  $P = 0.7$ , Wilcoxon signed rank test).

#### Effect of i.c.v. benoxathian on secretin-induced excitation of vasopressin neurons

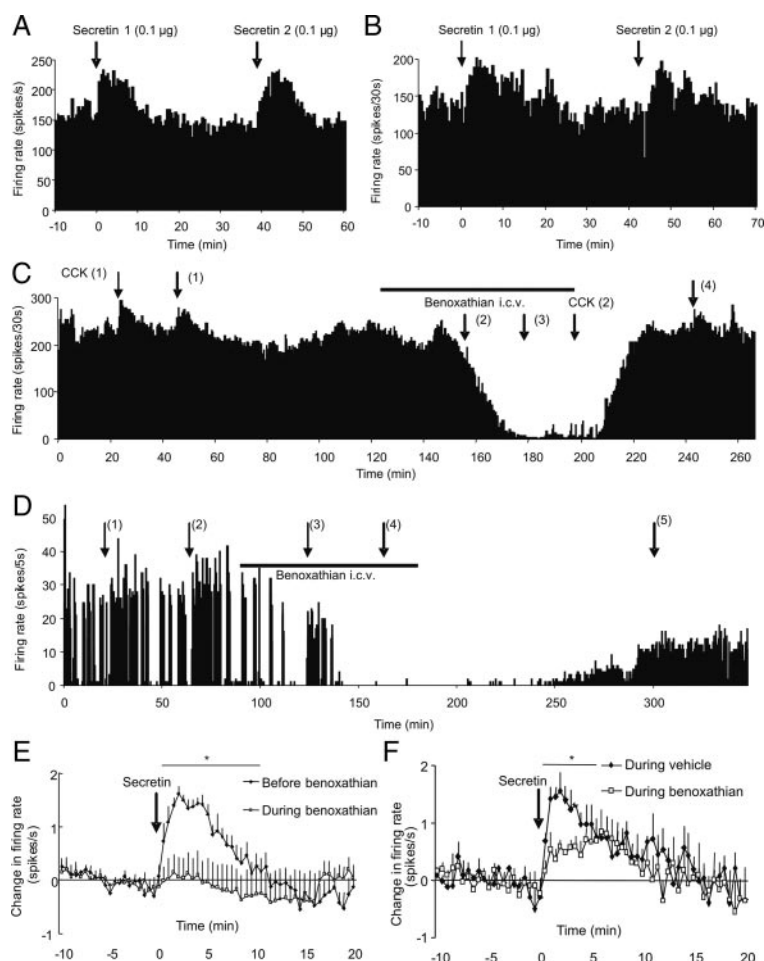
Benoxathian i.c.v. infusion inhibited three of four phasic vasopressin neurons within 20 min after the infusion was begun, and during benoxathian infusion, there was no effect of secretin on any of these three cells (e.g. Fig. 5D). After stopping the infusion, the cells gradually recovered their basal activity, but we did not see a recovery of the response to secretin within the recording period.

#### Effect of benoxathian microdialysis on secretin-induced excitation of oxytocin neurons

Three oxytocin neurons were tested during benoxathian microdialysis onto the SON. Benoxathian microdialysis alone did not significantly reduce the basal firing rate (average  $2.5 \pm 0.6$  spikes/sec before and  $1.7 \pm 0.5$  spikes/sec during benoxathian,  $P = 0.1$ , paired  $t$  test; the evident reduction was similar to a previous finding) (22) but attenuated the secretin-induced excitatory response in all three cells. During vehicle microdialysis, the firing rate was increased by  $1.6 \pm 0.3$  spikes/sec at 2 min after secretin, whereas during benoxathian microdialysis, the firing rate was increased by only  $0.6 \pm 0.1$  spikes/sec at the same time point (Fig. 5F;  $P = 0.03$ , paired  $t$ -test, between vehicle and benoxathian).

#### Discussion

Here we show that near-physiological systemic doses of secretin excited SON oxytocin neurons and increased oxytocin secretion. The excitatory action of secretin was similar to the response to CCK, but secretin was more potent. The effect of secretin on SON vasopressin neurons was



**FIG. 5.** Effect of benoxathian i.c.v. infusion on secretin-induced responses of oxytocin and vasopressin neurons in urethane-anesthetized female rats. Benoxathian infusion rate was  $8 \mu\text{g}/\mu\text{l min}^{-1}$ . The excitatory response to secretin ( $0.1 \mu\text{g}/\text{rat}$ ; iv) before benoxathian i.c.v. administration was compared with the response during benoxathian administration. A and B, Representative recordings of oxytocin and vasopressin neuron responses to repeated injections of secretin ( $0.1 \mu\text{g}$ ; iv) 38 and 40 min apart, respectively. Similar responses to the repeated dose of secretin ruled out receptor desensitization. C, Representative recording of the effect of i.c.v. benoxathian infusion on secretin-induced responses in a SON oxytocin neuron. CCK ( $25 \mu\text{g}/\text{kg}$ ; iv) was given before (CCK1; to identify the cell as an oxytocin neuron from the excitatory response) and during (CCK2) benoxathian infusion. Secretin ( $0.1 \mu\text{g}$ ; iv) was given before (1), during the infusion [(2) and (3)], and after stopping benoxathian infusion (4). Benoxathian inhibited basal firing rate and blocked CCK and secretin-induced responses; a response to secretin returned after benoxathian infusion was stopped. D, Responses of a vasopressin neuron to secretin during benoxathian infusion ( $8 \mu\text{g}/\mu\text{l min}^{-1}$ ; i.c.v.; black bar). Secretin ( $0.1 \mu\text{g}$ ; i.v., arrows) was given before [(1) and (2)], during [(3) and (4)], and after the infusion (5). Secretin given before the infusion increased the activity quotient [secretin (1): from 0.07 to 0.38; secretin (2): from 0.28 to 0.43] and the frequency within bursts [secretin (1): from 4.49 to 4.78 spikes/sec; secretin (2): from 4.63 to 5.73 spikes/sec] and decreased the mean interburst interval [secretin (1): from 69.6 to 34.6 sec; secretin (2): not calculated as only one burst occurred during the basal 5 min period]. Secretin (3) given 23 min after starting the infusion also elicited an excitatory response, but secretin (4) given 58 min after starting the infusion had no effect. The cell started to regain activity from 30 min after stopping the benoxathian infusion. Secretin (5) given 120 min after stopping the infusion elicited a slight excitatory response (activity quotient increased from 0.03 to 0.18 and frequency within bursts increased from 0.94 to 1.52 spikes/sec). E, Effects of i.c.v. benoxathian on oxytocin neurons. Values are group mean  $\pm$  SEM;  $n = 6$  cells/rats. Pre- vs. 0–10 min postsecretin before benoxathian infusion:  $P < 0.001$ , paired  $t$  test; pre- vs. 0–10 min postsecretin during benoxathian infusion:  $P = 0.7$ , Wilcoxon signed rank test; \*,  $P = 0.006$ , 0–5 min response to secretin before vs. during benoxathian infusion,  $t$  test. F, Responses of oxytocin neurons to secretin during benoxathian microdialysis: benoxathian was microdialyzed ( $2 \text{ mM}$ ;  $3 \mu\text{l}/\text{min}$ ) onto the ventral surface of the SON;  $n = 3$  cells/rats. Pre- vs. 0–10 min postsecretin during vehicle microdialysis:  $P = 0.03$ , paired  $t$  test; pre vs. 0–10 min postsecretin during benoxathian:  $P = 0.1$ , paired  $t$  test; 0- to 5-min response to secretin during vehicle vs. benoxathian, \*,  $P = 0.03$ ,  $t$  test.

predominantly excitatory, unlike CCK, which was inhibitory. Blockade of the effects of secretin by benoxathian, given by i.c.v. infusion or by microdialysis onto the SON, indicates that noradrenergic pathways are involved in the secretin-induced excitation of SON neurons, as previously found for CCK actions.

Previous studies have found that iv secretin ( $40 \mu\text{g}/\text{kg}$ ) and i.c.v. secretin ( $1\text{--}10 \mu\text{g}$ ) induce Fos in SON oxytocin neurones (4, 23). However, the present study shows that iv doses as low as  $0.1\text{--}1 \mu\text{g}$  significantly excite SON oxytocin and vasopressin neurons, and induce oxytocin secretion. From the responses of oxytocin neurons to secretin and CCK, it appears that secretin is far more potent than CCK. The secretin injection given in this study contained approximately 200-fold fewer moles of secretin ( $33 \text{ pmol}$ ) than the CCK ( $6.6 \text{ nmol}$ ) injection, and the iv dose of  $0.1 \mu\text{g}$  used in the present study is the lowest systemic dose reported to have central effects.

The magnocellular neurons of the SON all express either oxytocin or vasopressin. They also express, in much lower amounts, a profusion of other peptides including many appetite-related peptides, including apelin, cocaine and amphetamine regulated transcript, CCK, dynorphin, galanin, and neuropeptide Y, together with their cognate receptors (24). Most recently, it has been shown that vasopressin cells also express secretin and its receptor, and that at high concentrations ( $100 \text{ nM}$ ) secretin can stimulate vasopressin release from the isolated SON, and i.c.v. administration of  $0.45 \mu\text{g}$  secretin can cause vasopressin release into the circulation (21). Thus, secretin clearly is capable of directly activating vasopressin cells. However, in the present study the effects of lower doses of secretin given systemically ( $0.1 \mu\text{g}$  iv vs.  $0.45 \mu\text{g}$  i.c.v.) probably did not involve direct effects on the SON neurons as the effects were abolished by i.c.v. benoxathian. This finding is consistent with



the interpretation that the physiological actions of circulating secretin are on the afferent vagal nerve endings, which signal to the hypothalamus via noradrenergic pathways from the caudal brain stem. This conclusion is supported by finding that the half-lives of change in firing rate after administration of secretin (5.1 min) and CCK (3.8 min) corresponded with the estimated respective circulating half-lives of secretin ( $1.5$  to  $5 \pm 2$  min in rats) (19, 20) and CCK (1.5–3 min, measured in dogs) (25).

Interestingly, systemic secretin predominantly excited vasopressin neurons, in striking contrast with the actions of CCK, which either inhibits or has no effect on them (9–11). The passive movement of water from plasma into the intestinal lumen after secretin-induced exocrine pancreatic secretion of  $\text{HCO}_3^-$  results in plasma hyperosmolality and stimulates vasopressin release (26, 27). However, the rapid effects of secretin on vasopressin neurons suggest that an additional, more direct, secretin-induced activation is possible for SON vasopressin neurons. Both secretin and CCK transiently increase blood pressure after injection (28–30), so the activation of vasopressin neurons by secretin is not a consequence of decreased blood pressure.

Systemic secretin activates vagal afferents (6) and depolarizes neurons in the NTS (4). Subdiaphragmatic vagotomy abolishes Fos expression induced by secretin in the NTS, but it was maintained in the area postrema (AP) (4) suggesting that secretin may act directly on the AP neurons. The SON receives excitatory noradrenergic projections from the NTS. The AP also has efferent projections to the SON via the A1 cell group (31, 32). The AP and NTS have reciprocal connections; hence, afferent input to the AP may be relayed first to the NTS and then conveyed to the SON or *vice versa* (33). Thus, the mode of action of systemic secretin might be either direct on secretin receptors on the SON neurons (5), which would require secretin crossing the blood-brain barrier, or indirect through activating vagal afferents and subsequently the brain stem nuclei. Central infusion of benoxathian significantly attenuated the basal firing and secretin-induced excitation of oxytocin neurons indicating that central noradrenergic pathways mediate the secretin-induced excitation of oxytocin neurons via  $\alpha$ -1 adrenoceptors. A similar inference can be drawn for vasopressin neurons, as i.c.v. benoxathian also abolished secretin-induced excitation of vasopressin neurons.

Systemic administration of CCK activates gastric vagal afferents, resulting in the activation of NTS neurons, which then excites SON oxytocin neurons via excitatory noradrenergic projections from the NTS (9, 10). Although the findings for oxytocin neurons are consistent with actions of circulating secretin and CCK via a noradrenergic

projection from the NTS, the opposite actions of iv secretin (excitatory) and CCK (inhibitory) on vasopressin neurons would seem not to be explained by such a possibly common pathway.

Secretin, oxytocin, and vasopressin have intertwined regulatory roles in gastric motility, water and electrolyte homeostasis, and energy homeostasis (7, 24, 34–38). The excitation of SON oxytocin and vasopressin neurons by near-physiological levels of secretin in this study suggests that some physiological effects of secretin might be mediated through secretion of these neurohypophysial hormones. A physiological role of the endocrine secretin signal that food has entered the small intestine may be that the stimulation of oxytocin and vasopressin secretion leads to excretion of ingested salt and conservation of water, after water movement into the intestine, hence maintaining homeostasis.

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